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Detection of Candida Albicans Virulence Genes Isolated from Periodontitis Patients in Salah Al-Din City

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Abstract:*This study aimed to isolate and diagnose the Candida albicans in Periodontitis Patients visiting the consulting clinic at Salah al-Din General Hospital and some private clinics and diagnosed by doctors during the period October 2024 to January 2024 from patients. A total of 75 clinical samples were collected during this period. Patients between the ages of 5 and 55, who were diagnosed with periodontitis and received treatment at a specific dental health facility and outpatient clinics of dental in Salah al-Din City, Iraq.*
Materials and method: *The first diagnosis is achieved by direct microscopic examination, whereas the second method uses laboratory culture on Sabouraud's Dextrose Agar (SDA) medium and biochemical yeast assays (urease test). The swab was cultured on CHROM and SAD to isolate C. albicans. Once validated, genomic DNA was extracted from the colony. Polymerase chain reaction discovered ALS1 and HWP1 virulence genes.*
Results: *The microscopic analysis revealed that out of the total number of samples (75), 55 samples tested positive, accounting for a rate of 27%. The second method involves analyzing the results of laboratory culture using Sabroid Dextrose Agar (SDA) medium. The analysis revealed that 40 swabs tested positive, On the other hand, 35 swabs tested negative and did not show any growth, accounting for 47% of the samples.Statistical study revealed substantial variations in the ability of isolated yeasts to produce hemolysin, with a p-value of 0.0006. The analysis revealed that all 75 samples (100%) were identified as C. albicans. Furthermore, the study found that the ALS1 gene was detected in 18 samples (72%), while the HWP1 gene was recognized in 15 samples (60%).*

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Conclusion: The majority of C. albicans isolates exhibited the HWP1 and Als1 virulence genes, suggesting that the ALS1 and HWP1 proteins play a crucial role in the development of infection.

Keywords: Als1 Gene, Candida Albicans, Hwp1 Gene, Pcr, Periodontist.

1. INTRODUCTION

Periodontitis is clinically characterized by the loss of attachment around teeth, resulting in the formation of periodontal pockets and the deterioration of bone.[1] The oral cavity serves as an indicator of general well-being and is susceptible to various adverse reactions caused by drug use. Multiple studies have established a link between Candida spp. yeasts and their possession of numerous virulence factors, which aid in their ability to disseminate and infect different areas of the human body [2]. Virulence factors are a measure of the extent of pathogenicity of a pathogen, as it is not capable of causing infection unless it is virulent. These factors may be in the form of cellular structures, enzymes, or mycotoxins that facilitate the infection process and protect the pathogen from immune responses and various surrounding conditions. The virulence factors in yeasts of the Candida genus are summarized in the property of adhesion, which is the first step in causing infection, followed by polymorphism, phenotypic change, and the formation of chlamydial spores, Chlamydospores, hemplysin, the formation of the germ tube, which occurs under certain conditions, and the production of secreted hydrolytic enzymes that facilitate the invasion process. And penetration by contributing to the analysis of peptide bonds that connect the plasma membrane proteins of host cells [3].

Virulence factors are a measure of the extent of pathogenicity of a pathogen, as it is not capable of causing infection unless it is virulent. These factors may be in the form of cellular structures, enzymes, or mycotoxins that facilitate the infection process and protect the pathogen from immune responses and various surrounding conditions. The virulence factors in yeasts of the Candida genus are summarized in the property of adhesion, which is the first step in causing infection,

2. RELATED WORKS

Candida species have various potential virulence factors, such as secreted aspartyl proteinases, phospholipases , cell wall glycoproteins (adhesins), ALS gene,and hyphal wall protein HWP. Candida albicans possesses several characteristics that facilitate its adherence to diverse targets, such as cells of other microbes, inanimate surfaces, and numerous host cells. [4,5]. The development of systemic candidiasis requires the expression of many genes, including ALS1, ALS3, ECE1, and HWP1 [6].

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pathogen from immune responses and various surrounding conditions. The virulence factors in yeasts of the Candida genus are summarized in the property of adhesion, which is the first step in causing infection, Specifically, the gene HWP1 is responsible for encoding a prominent protein in C. albicans that plays a crucial role in various processes, including the formation of the cell wall, intracellular communication, and the growth of hyphae. This protein achieves these functions by forming connections with the glucans present in the cell wall[7]. In addition, HWP1 enhances the adherence of Candida to epithelial cells, which is the initial step in colonization and it augments the severity of systemic candidiasis [8].

Aim of Study

To achieve the goal, the following steps were followed:
1-Isolating the yeasts that cause Periodontitis in patients, diagnosing them using biochemical tests.
2-Detection of some virulence factors of yeasts and their ability to produce hemolysin, cell wall glycoproteins (adhesions), ALS gene,and HWP gene.

3. METHODOLOGY

Materials and Method

75 clinical samples were collected during the period October 2024 to January 2024 from Periodontitis patients. They were collected from Salah al-Din General Hospital and some private clinics. Samples were collected for patients with Periodontitis whose ages ranged from (5-55). The initial diagnosis and examination for follow-up examinations were conducted with the assistance of the gynecologist. Samples were collected from Periodontitis using sterile cotton swabs from the moth. Samples were kept in special sterile containers until they were transported to the laboratory to conduct the necessary tests.

Laboratory Examination of Samples

Direct Microscopic Examination

A portion of the sample taken by swab cotton was placed on a glass slide, and a drop of 10% KOH potassium hydroxide solution was added to it, then it was covered with a cover slide and then passed over the flame two or three times, after which it was examined with a microscope. Under 10X power and then 40X power in order to confirm the presence of yeast.

Macroscopic Examination

The external appearance of the colonies growing on sabroid dextrose agar (SDA) medium was examined, in terms of colour, texture, odor, and the shape of the colony from both the plate and diameter sides [9].

Virulence Factors

Hemolysin Test

This test was conducted according to Jabber et al. (2015), to detect the ability of yeasts to analyze blood, sterile sabroid dextrose agar SDA medium was used and 7% sheep blood was added to it. Part of the colony was transferred to the dish using the planning method. The

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dishes were incubated at a temperature of 37°C for 24 hours. The results were recorded. Based on observing the decomposition area around the colony [10].

DNA Extraction

The DNA was obtained via the Geneaid kit. As per the manufacturer's instructions, 4-6 colonies of yeast were combined with 1.5 mL of sterilized 0.9% NaCl. After subjecting the microtubes to centrifugation at a force equivalent to 1,216,000 times the acceleration due to gravity for a period of 2 minutes, the liquid fraction containing the desired compounds was collected and then washed with a solution of 2-millimolar EDTA at a pH of 8. After subjecting the microtubes to centrifugation at a force equivalent to 5000 times the acceleration due to gravity for a period of 10 minutes, separate the liquid portion above the sediment and save the solid residue. The nanodrop spectrophotometer quantifies the concentration and assesses the quality of DNA by detecting absorption at 260/280 nm.

Primer and the Polymerase Chain Reaction With Uniplex Units

Using a primer from Table 1, uniplex polymerase chain reaction (PCR) detected molecules.

Table 1: The conditions of the primer and the amplification pathogenicity genes of Candida albicans pathogen

C albicans Genes	Sequence of Primer (5' -------- 3')	PCR amplicon size
ALS1 F R	GACTAGTGAACCAACAAATACCAGA CCAGAAGAAACAGCAGGTGA	318
HWP1 F R	ATGACTCCAGCTGGTTC TAGATCAAGAATGCAGC	503

The Mixture For Polymerase Chain Reaction

The procedure used by PCR to find the C. albicans genes was carried out in a finished volume that is shown in Table 2.

Table 2: reaction volume for the PCR

Content	Volume (ml)
Master Mix	7.5
DNA template	5
Forward Primer	2.5
Reverse Primer	2.5
Nuclease Free Water	2.5
Total	20

Polymerase Chain Reactionthermocycler Program

The PCR settings were employed to identify the pathogenic genes of C. albicans, as demonstrated in Table 1. Subsequently, the presence of the PCR amplicon was verified using agarose gel electrophoresis with a concentration of 1.5% agarose.

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Statistical Analysis

The statistical study of the data was done with a piece of software called SPSS, which stands for Statistical Program for the Social Sciences. An version of the Pearson chi-square test was used to figure out how different the groups were from each other.

Table 3: PCR detection of the proportion of the C. albicans pathogenicity gene.

Gene	Positive Number	Negative Number	Total
C. albicans ALS1	18 (72 %)	7(28 %)	25(100%)
C. albicans HWP1	15 (60 %)	10(40 %)	25(100%)

4. RESULTS AND DISCUSSION

Samples of Yeasts Isolated During the Study

The results of the microscopic examination of the total number of samples (75) showed that there were (55) positive samples, at a rate of 73%, while the number of negative samples was 20, at a rate of 27%. As for the results of laboratory culture after culturing the samples on sabroid dextrose agar (SDA) medium, they showed (40) of the swabs were positive, at a rate of 53%, while (35) of the swabs were negative, showing no growth, at a rate of 47%, as in Table (3). The results of the statistical analysis showed that there were no significant differences, P-Value = 0.122.

Table 3: Numbers and percentages for direct microscopic examination and laboratory culture of samples

%	Negative samples	%	Positive samples	n.	Type of examination
%27	20	%735	55	75	Direct microscopic examination
%47	35	%453	40	75	Laboratory culture
P-Value = 0.122		Chi-Square = 0.881 ns			

The reason for the appearance of negative results and the difference in results is due to the similarity in symptoms between candidiasis and bacterial or viral infections, the random use of antibiotics without consulting a doctor, or the inadequacy of the sample collected. It can also be attributed to inappropriate development conditions on the agricultural medium (SAD) [11]. The increase in the prevalence of vaginal candidiasis among women is due to lack of attention to personal hygiene, lack of awareness, poor nutritional habits, wearing tight underwear for some women, and lack of effective treatment [12]. Hence, clinical diagnosis alone lacks the necessary precision for accurate diagnosis. It must be complemented by other diagnostic methods, such as direct microscopic examination. This method yields more precise results compared to clinical examination, as it relies on the detection of yeast cells, fungal hyphae, or any other component of the pathogen, making it a more accurate approach [13].

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Figure 1: Candida albicans colonies on Sabouraud chloramphenicol media

Figure 2: of C. albicans morphology on CHROM agar

Detection of the Ability of C.Albicans on The Production of Hemolysin

The results of the study, shown in Table (4), showed that the ability of Candida to decompose blood. The yeast C.albicans had the ability to decompose blood at a rate of 45%, with a rate of 34 out of 75, while rate of 55%, with a rate of 41 out of 75did not have the ability to lyse blood. The results of the statistical analysis showed that there were significant differences, p-value=0.0006, regarding the ability of Candidia spp. on the production of hemolysin.

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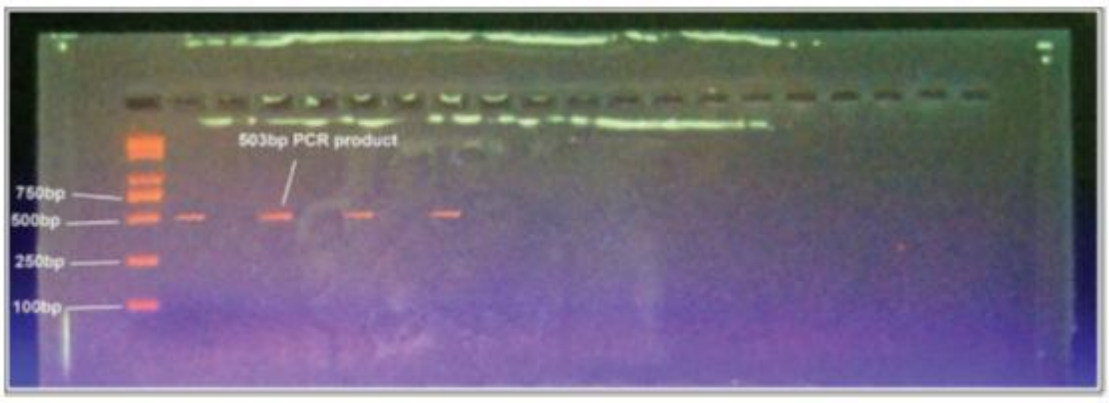
Table 4: Detection of the ability of Candidia spp. on the production of hemolysin

%	non production of hemolysin	%	production of hemolysin	the total number	Candida spp.
%55	41	%45	34	75	C.albicans
P-Value =0.0006			Chi-Square = 19.10 **		

Our results agreed with Jacob and D'Souza (2014), with regard to the species C. albicans produce hemolysin [14]. As well as our results agreed with the results of the researcher Haciog et al., (2019), Who showed that C.albicans produce hemolysin while C.parapsilosis does not produce hemolysin [15].

Figure 3: The PCR amplicon of ALS1 genes (318 base pairs) within C. albicans was subjected to gel electrophoresis on a 1.5% agarose gel at a voltage gradient of 7 volts per centimeter for a duration of 30 minutes. Lane 1 has a DNA fragment that is 1000 base pairs long.

Figure 4: The PCR amplification, consisting of HWP1 genes with a length of 503 base pairs, was analyzed using gel electrophoresis. The electrophoresis was conducted on a 1.5% agarose gel with a voltage gradient of 7 volts per centimeter for a period of 30 minutes. Lane 1 has a DNA fragment with a length of 1000 base pairs.



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Periodontal disease is a complex inflammatory illness that impacts the tissues that provide support to the tooth and is linked to other inflammatory disorders in the body. The distinction between C. albicans and other Candida spp. was established by analyzing their morphological characteristics, such as colony shape, size, color, borders [16,17]. A smooth and creamy white appearance can be observed on the C. albicans colony that is grown on the Sabouraud medium[18]. A higher prevalence of Candida albicans was detected in our analysis when compared to the rates reported by Sardi et al.[19] in Iraq. They discovered that the rates of Candida albicans isolated from oral infections were 8 (7.4%) out of a total sample size of 108. Our investigation demonstrated that the prevalence of Candida albicans was higher.

Discussion

There was a striking similarity between the molecular discovery of the ALS1 gene and the research carried out by Ali et al.[20], in which they effectively isolated the ALS1 gene in 12 out of 25 samples. Unlike the findings of Mohammed et al.[21] in their study conducted in Iraq, the present investigation produced contrasting results. More precisely, it was noted that none of the C. albicans strains obtained from oral and vaginal infections exhibited a positive result for ALS1. [22,23]. Moreover, scientific research has demonstrated that the virulence factors HWP1 and SAP4 play a critical role in the formation of biofilms, which can harm the host's tissues and promote the growth of hyphae[24].

Reports indicate that the HWP1 gene facilitates the attachment of C. albicans to epithelial cells via covalent bonding. Prior studies have demonstrated that the interaction between the ALS1/3 and HWP1 surface protein is essential for the onset and advancement of hemolysin production. Previous research has proposed that the ALS1, ALS3, and HWP1 genes have complementary roles in the synthesis of hemolysin [25].

5. CONCLUSION

1. Isolation of C.albicans species ranked first among the other isolated species from Periodontitis patients.
2. C.albicans was shown to be capable of producing hemolysin
3. The majority of C. albicans isolates exhibited expression of the Als1 and HWP1 virulence genes, indicating that the ALS1 and HWP1 proteins had a significant impact on the development of infection.

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